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Characterization of the Palmitoylation Domain of SNAP-25

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Abstract: SNAP-25 (synaptosomal associated protein of 25 kDa) is a neural specific protein that has been implicated in the synaptic vesicle docking and fusion process. It is tightly associated with membranes, and it is one of the major palmitoylated proteins found in neurons. The functional role of palmitoylation for SNAP-25 is unclear. In this report, we show that the palmitate of SNAP-25 is rapidly turned over in PC12 cells, with a half-life of ~3 h, and the half-life for the protein is 8 h. Mutation of Cys to Ser at positions 85, 88, 90, and 92 reduced the palmitoylation to 9, 21, 42, and 35% of the wild-type protein, respectively. Additional mutations of either $Cys^{85,88}$ or $Cys^{90,92}$ nearly abolished palmitoylation of the protein. A similar effect on membrane binding for the mutant SNAP-25 was observed, which correlated with the degree of palmitoylation. These results suggest that all four Cys residues are involved in palmitoylation and that membrane association of SNAP-25 may be regulated through dynamic palmitoylation. **Key Words:** Synaptic proteins—SNAP-25—Palmitoylation—Membrane association—Mutagenesis—Protein turnover.
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The synaptosomal associated protein of 25 kDa, SNAP-25, is a member of the synaptic vesicle docking/fusion complex that includes syntaxin, synaptobrevin (VAMP), and synaptotagmin (for reviews, see Bennett and Scheller, 1994; Ferro-Novick and Jahn, 1994; Rothman and Warren, 1994; Südhof, 1995). It has been shown to interact with both syntaxin and synaptobrevin by in vitro binding assays (Chapman et al., 1994; Pevsner et al., 1994; McMahon and Südhof, 1995). Analysis of cDNA clones of SNAP-25 from mouse (Oyler et al., 1989), chicken (Catsicas et al., 1991), and *Drosophila* and *Torpedo* (Risinger et al., 1993) showed that the protein is highly conserved through evolution and is expressed specifically in the nervous systems. SNAP-25 plays an important role in synaptic vesicle docking/fusion. Specific cleavage of the protein by botulinum neurotoxins A and E blocks neurotransmitter release at neuromuscular junctions (Blasi et al., 1993; Schiavo et al., 1993; Binz et al., 1994). More recently, SNAP-25 homologues from yeast and nonneuronal tissues were cloned, which showed high degrees of homology in the important functional domains (Brennwald et al., 1994; Ravichandran et al., 1996). These results strongly suggest that SNAP-25 and its homologues play fundamental roles in vesicular transport.

SNAP-25 is localized primarily to the membrane fractions in both developing and adult brains (Oyler et al., 1989). It is one of the major palmitoylated proteins found in neurons (Hess et al., 1992), and this post-translational modification may play an important role in modulating the physiological functions of the protein. Deletion of a 12-amino acid sequence containing the four Cys residues of SNAP-25 abolished palmitoylation and membrane association of the protein, suggesting that palmitoylation may be required for membrane association (Veit et al., 1996). Two SNAP-25 isoforms generated by alternate splicing are expressed in nervous systems. The isoforms, designated as SNAP-25*a* and *b*, differ in a nine amino acid sequence containing the putative palmitoylated Cys residues (Bark et al., 1995). It has been shown that the two isoforms are differentially sorted into the neurites in PC12 cells, suggesting that palmitoylation may modulate the sorting of SNAP-25 into the neuronal processes (Bark et al., 1995). It is, therefore, important to understand the regulation and the functional significance of SNAP-25 palmitoylation. In the present report, we have characterized the palmitoylation domain of SNAP-25 by using site-directed mutagenesis. Our results showed that SNAP-25 is rapidly palmitoylated and depalmitoylated, which may provide a regulatory mechanism for its tight membrane association.

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Abbreviations used: DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; PEI, polyethylenimine; SDS, sodium dodecyl sulfate; SNAP-25, synaptosomal associated protein of 25 kDa; 85S, SNAP-25 in which Cys^{85} is substituted with Ser; 88S, SNAP-25 in which Cys^{88} is substituted with Ser; 90S, SNAP-25 in which Cys^{90} is substituted with Ser; 92S, SNAP-25 in which Cys^{92} is substituted with Ser; 85,88S, SNAP-25 in which $Cys^{85,88}$ are substituted with Ser; 90,92S, SNAP-25 in which $Cys^{90,92}$ are substituted with Ser; 85,88A, SNAP-25 in which $Cys^{85,88}$ are substituted with Ala; 90,92A, SNAP-25 in which $Cys^{90,92}$ are substituted with Ala.

EXPERIMENTAL PROCEDURES

Plasmid construction and mutagenesis

The cDNA encoding the mouse SNAP-25*b* (pSNAP8.52, Oyler et al., 1989; a generous gift from Dr. Michael C. Wilson, Scripps Research Institute, San Diego, CA, U.S.A.) was cloned into the expression vector pcDNA3 (Invitrogen) at the *Kpn*I and *Xba*I sites. For site-directed mutagenesis of 85S (SNAP-25 in which Cys^{85} is substituted with Ser), 88S (SNAP-25 in which Cys^{88} is substituted with Ser), 90S (SNAP-25 in which Cys^{90} is substituted with Ser), 92S (SNAP-25 in which Cys^{92} is substituted with Ser), 85,88S (SNAP-25 in which $Cys^{85,88}$ are substituted with Ser), and 90,92S (SNAP-25 in which $Cys^{90,92}$ are substituted with Ser), a *Hin*dIII and *Eco*RV restriction fragment of pSNAP8.52 was subcloned into pSP72 vector to generate pSP72SNAP-25. Mutagenesis was performed by polymerase chain reaction (PCR) using *pfu* DNA polymerase (Stratagene). The primers were as follows: upstream: 5′-GTC GCA TGC TGC AGC TGG TCG A-3′; downstream: 85S, 5′-TTT AAG CTT GTT ACA GGG ACA CAC ACA AAG CCC GCT GAA-3′; 88S, 5′-TTT AAG CTT GTT ACA GGG ACA CAC ACT AAG-3′; 90S, 5′-TTT AAG CTT GTT ACA GGG ACT CAC A-3′; 92S, 5′-TTT AAG CTT GTT ACT GGG ACA CAC A-3′; 85,88S, 5′-TTT AAG CTT GTT ACA GGG ACA CAC ACT AAG CCC GCT GAA-3′; 90,92S, 5′-TTT AAG CTT GTT ACT GGG ACT CAC A-3′. The resulting PCR products were digested with *Hin*dIII and *Pst*I, and were cloned into the pSP72SNAP-25. The *Hin*dIII and *Eco*RV fragment with the mutations was then cloned into the pSNAP8.52 and the cDNAs encoding the full-length mutant SNAP-25 were cloned into the pcDNA3 at the *Kpn*I and *Xba*I sites. For mutagenesis of 85,88A (SNAP-25 in which $Cys^{85,88}$ are substituted with Ala) and 90,92A (SNAP-25 in which $Cys^{90,92}$ are substituted with Ala), the wild-type SNAP-25 in pcDNA3 was used as a template for PCR mutagenesis using the Quickchange kit (Stratagene). The primers were as follows: 85,88A: 5′-TA GGA AAA TTC GCC GGG CTT GCT GTG TGT CCC T-3′ and 5′-A GGG ACA CAC AGC AAG CCC GGC GAA TTT TCC TA-3′; 90,92A: 5′-GG CTT TGT GTG GCT CCC GCT AAC AAG CTT A-3′ and 5′-T AAG CTT GTT AGC GGG AGC CAC ACA AAG CC-3′. All the mutations were confirmed by DNA sequencing analysis.

Cell culture and transfection

COS-7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and maintained in a humidified 35°C incubator with 5% CO_2. Transient expression of wild-type and mutant SNAP-25 was accomplished using the polyethylenimine (PEI) protocol (Boussif et al., 1995). In brief, the cells were plated at ~70% confluency the day before transfection. The cells were rinsed once with serum-free DMEM, and incubated in serum-free DMEM with 20 μg DNA and 10 m*M* PEI at a DNA-to-PEI ratio of ~10. The cells were incubated for 3–5 h before FBS was added to a final concentration of 10%. Forty-eight hours after transfection, the cells were collected and homogenized on ice with homogenization buffer containing 10 m*M* Tris-HCl, pH 7.5, 1 m*M* EDTA, and 0.5 m*M* phenylmethylsulfonyl fluoride. The homogenates were then centrifuged at 150,000 *g* for 1 h at 4°C, and the total particulate and the cytosolic fractions were collected. The pellets were resuspended by sonication with the same volume of homogenizing buffer containing 0.1% sodium dodecyl sulfate (SDS). Equal aliquots of the cytosolic and membrane fractions were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis.

Metabolic labeling and immunoprecipitation

For in vivo labeling with [^{3}H]palmitic acid, COS-7 cells were transfected as described above. The cells were incubated in serum-free DMEM for 1 h and then labeled with 0.5 mCi/ml [^{3}H]palmitic acid (NEN) in the same media for 3 h. The labeled cells were lysed by sonication in 10 m*M* Tris-HCl, pH 7.5, 1 m*M* EDTA, and 1% SDS. The cell lysates were then diluted in 1% Nonidet P-40, 100 m*M* NaCl, 50 m*M* Na_2HPO_4, pH 7.4, to make the final SDS concentration of 0.1%.

Immunoprecipitation of SNAP-25 was performed by using protein G plus agarose (Oncogene Science) and a goat anti-SNAP-25 antibody raised against a glutathione-*S*-transferase–SNAP-25 fusion protein (Y. Liu, unpublished result). The immunocomplex was pelleted by centrifugation and washed twice with 50 m*M* Na_2HPO_4, pH 7.4, and 100 m*M* NaCl. The immunoprecipitated proteins were heated at 70°C for 5 min in 2% SDS, 1 m*M* EDTA, 1 m*M* dithiothreitol, 15% glycerol, 20 m*M* Tris-HCl, pH 6.8, and 0.01% bromophenol blue. The labeled proteins were analyzed by SDS-PAGE and fluorography. For determining the turnover rate of palmitate and SNAP-25, PC12 cells were preincubated in either DMEM or Met/Cys-free DMEM without serum for 1–2 h. The cells were then labeled in the same media with either 0.7 mCi/ml [^{3}H]palmitic acid or 100 μCi/ml Tran^{35}S-label (ICN) for 3–20 h. After washing the cells with phosphate-buffered saline, the labeled cells were chased in DMEM supplemented with 10% FBS and 100 μ*M* palmitate for ^{3}H-labeled cells, and in DMEM supplemented with 10% FBS for ^{35}S-labeled cells. SNAP-25 was immunoprecipitated and analyzed as described above.

RESULTS

SNAP-25 undergoes dynamic palmitoylation and depalmitoylation

Many proteins are reversibly palmitoylated. The half-lives of the palmitates for certain proteins ranged from 90 to 120 min (Wedegaertner and Bourne, 1994; Schweizer et al., 1996). Such rapid turnovers may be important in regulating the functions of these proteins. The turnover rate for the palmitate and the polypeptide of SNAP-25 is not known. Because both SNAP-25*a* and *b* isoforms are expressed in PC12 cells, with the SNAP-25*a* as the predominant species (Bark et al., 1995), PC12 cells were used to determine the turnover rate for the palmitic acids of SNAP-25. The cells were labeled with [^{3}H]palmitic acid for 3 h and chased for up to 17 h. The palmitoylated SNAP-25 was analyzed by immunoprecipitation and SDS-PAGE fluorography. As shown in Fig. 1, the covalently bound [^{3}H]-palmitate was rapidly turned over. The half-life of the [^{3}H]palmitate was estimated to be ~3 h (Fig. 1). To determine the turnover rate of the SNAP-25 polypeptide, PC12 cells were labeled with [^{35}S]methionine for 3 h or overnight, and the cells were chased for up to 24 h. Both the shorter and longer labeling experiments

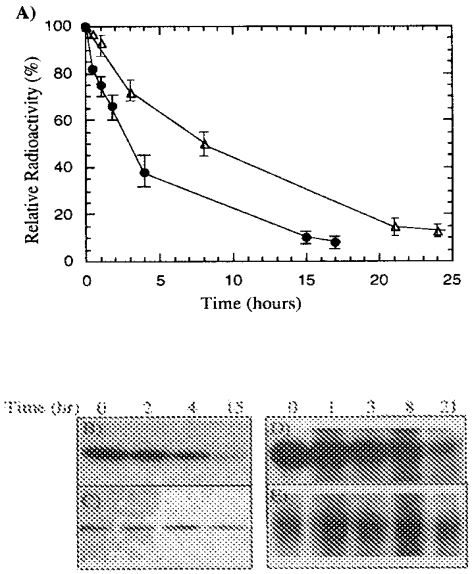

FIG. 1. Half-life of SNAP-25 and palmitate bound to SNAP-25 in PC12 cells. PC12 cells were labeled with 0.5 mCi/ml [^{3}H]palmitic acid for 3 h or 0.1 mCi/ml [^{35}S]methionine for 2 h as described in Experimental Procedures. The cells were washed once with DMEM and chased for various time periods as indicated. SNAP-25 was immunoprecipitated and divided into two aliquots for SDS-PAGE and immunoblot analysis. **A:** Turnover of the SNAP-25 protein (△) and palmitate (●). **B:** Fluorograph of immunoprecipitated SNAP-25 labeled with [^{3}H]palmitic acid. **C:** Immunoblot analysis of samples from B. **D:** Fluorograph of immunoprecipitated SNAP-25 labeled with [^{35}S]methionine. The control sample without primary anti-SNAP-25 antibody had no protein bands at the SNAP-25 position. **E:** Fluorograph of a 55-kDa polypeptide in the cell lysate as a control for [^{35}S]methionine labeling. Error bar = SEM. The values represent two independent experiments.

showed that the protein had a half-life of ~8–10 h, which is significantly shorter than the 37-h half-life of synaptophysin in PC12 cells (Green and Kelly, 1992). These results suggested that both the palmitates and the polypeptide of SNAP-25 were rapidly turned over in PC12 cells.

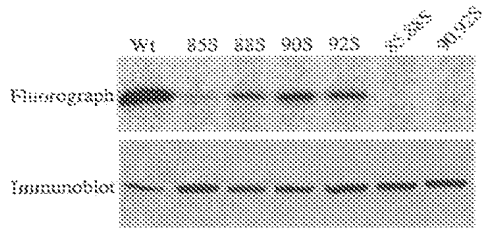

FIG. 2. Palmitoylation of wild-type and mutant SNAP-25 expressed in COS-7 cells. COS-7 cells were transfected and labeled with [^{3}H]palmitic acid as described in Experimental Procedures. The proteins were immunoprecipitated, and equal aliquots of the immunocomplex were analyzed by SDS-PAGE fluorography and immunoblot analysis. The figure is representative of three independent experiments.

All four Cys residues are important for palmitoylation

It has been shown that most of the palmitoylation occurs on the Cys amino acid. The four Cys residues of SNAP-25 are clustered between amino acids 85 and 92 (Table 1). Deletion of this sequence abolished palmitoylation of the protein (Veit et al., 1996). To address the role of the individual Cys residue, mutants in which each Cys was replaced with a Ser residue were prepared and expressed in COS-7 cells. As shown in Fig. 2, each Cys-to-Ser mutation led to a significant reduction in the incorporation of [^{3}H]palmitate into SNAP-25. The amount of incorporated [^{3}H]palmitate was determined by densitometry measurement of the x-ray film. Compared with the wild-type SNAP-25, 9% of [^{3}H]palmitate was incorporated in the 85S mutant, 21% for 88S, 42% for 90S, and 35% for 92S (Table 2). These results suggested that each of the four Cys were involved in the palmitoylation of SNAP-25, with the Cys^{85} as the most critical residue. Furthermore, the palmitoylation of the Cys appeared to be highly dependent on the other Cys residues. Mutation of one Cys greatly affected the palmitoylation of the other Cys. This was most evident for 85S, which reduced palmitoylation by >90%.

To further investigate the importance of the Cys residues, the rate of palmitoylation and depalmitoyla-

TABLE 1. *SNAP-25 mutants*

	85	88	90	92
Wt-*a*	Cys	Phe Ile		
Wt-*b*	Leu Gly Lys Phe Cys Gly Leu Cys Val Cys Pro Cys Asn Lys Leu Lys			
85S	Ser			
88S		Ser		
90S			Ser	
92S				Ser
85,88S	Ser	Ser		
90,92S			Ser	Ser
85,88A	Ala	Ala		
90,92A			Ala	Ala

Wt, wild type.

TABLE 2. *Palmitoylation and membrane association of SNAP-25 mutants*

SNAP-25 construct	Description	% of palmitoylation[a]	% in membrane[b]
Wt	Wild type	100	84 ± 3
85S	Cys^{85} to Ser^{85}	9 ± 2	14 ± 6
88S	Cys^{88} to Ser^{88}	21 ± 5	18 ± 4
90S	Cys^{90} to Ser^{90}	42 ± 5	28 ± 6
92S	Cys^{92} to Ser^{92}	35 ± 6	29 ± 7
85,88S	$Cys^{85,88}$ to $Ser^{85,88}$	ND	<8
90,92S	$Cys^{90,92}$ to $Ser^{90,92}$	<3	<8
85,88A	$Cys^{85,88}$ to $Ala^{85,88}$	ND	<8
90,92A	$Cys^{90,92}$ to $Ala^{90,92}$	ND	<8

ND, not detected.

[a] Incorporation of [^{3}H]palmitate in the SNAP-25 mutants was quantitated by laser densitometry of the fluorographs.

[b] Scanned immunoblot images measured using NIH Image 1.61. The values represent the percentages in total cellular extracts.

tion of 90S was compared with that of the wild-type protein in COS-7 cells. As shown in Fig. 3, the rates of palmitoylation for the wild-type and 90S SNAP-25 did not differ significantly. However, the rate for depalmitoylation by 90S was greatly accelerated (Fig. 3). These results suggested that the mutation might have affected the stability of the palmitates covalently attached to the protein. Mutation of additional Cys, such as $Cys^{85,88}$ to $Ser^{85,88}$, and $Cys^{90,92}$ to $Ser^{90,92}$, nearly abolished the incorporation of palmitic acid (Fig. 2, Table 2). To demonstrate that this was not simply due to the Cys-to-Ser mutation that increased slightly the polarity of the palmitoylation domain, two other mutants were also prepared. The 85,88A and 90,92A, in which the $Cys^{85,88}$ or $Cys^{90,92}$ were replaced with Ala residues, showed similar results of palmitate incorporation (Table 2). These results suggested that the presence of the Cys residues was crucial for palmitoylation of the protein, and all four Cys could be potentially palmitoylated.

Palmitoylation is required for membrane association of SNAP-25

It has been shown that SNAP-25 is tightly associated with membranes (Oyler et al., 1989). Because the protein is highly hydrophilic and has no classic membrane spanning/insertion domains, it was postulated that palmitoylation may provide a hydrophobic moiety for the protein (Hess et al., 1992). Additional association between SNAP-25 and membranes may come from its interaction with syntaxin that is inserted into the membranes. A previous report showed that deletion of a 12-amino acid sequence containing the Cys residues abolished both palmitoylation and membrane binding (Veit et al., 1996). To address directly the role of palmitoylation in membrane association of SNAP-25, we examined the distribution of the Cys mutants expressed in COS-7 cells. Membrane and cytosolic fractions were prepared and analyzed by immunoblot analysis. As shown in Fig. 4, mutations of the Cys greatly affected membrane binding of the protein. The extent of membrane association correlated well with the degree of palmitoylation of the mutants.

It was noticed during subcellular fractionation that the electromobility of the membrane-associated SNAP-25 was sometimes shifted slightly (Fig. 4). This was probably due to the palmitoylation of the polypeptide, which would increase the molecular mass of the protein. Such a mobility shift was also observed during pulse–chase labeling of newly synthesized SNAP-25 in PC12 cells (data not shown). These results demonstrated that palmitoylation is required for membrane association.

DISCUSSION

In this report we have shown that, in PC12 cells, the covalently bound palmitate of SNAP-25 was rapidly

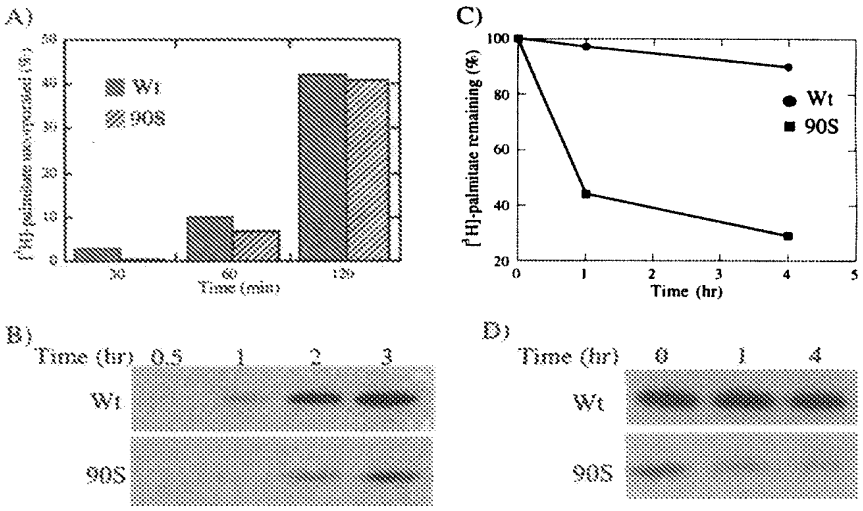

FIG. 3. Comparison of the palmitoylation and depalmitoylation rates of wild-type and 90S mutant SNAP-25. **A** and **B:** Determination of palmitoylation rates. COS-7 cells were transfected with the wild-type and 90S SNAP-25 and labeled with [^{3}H]palmitate for the indicated times. The cells were lysed immediately, and SNAP-25 was immunoprecipitated and analyzed by SDS-PAGE and fluorography. The incorporation of [^{3}H]-palmitate after 3 h of labeling was used as 100%. **C** and **D:** Determination of depalmitoylation rates. COS-7 cells were transfected and labeled for 3 h with [^{3}H]-palmitate and chased for up to 4 h as described in Fig. 1. Quantitation was performed using NIH Image 1.61ppc.

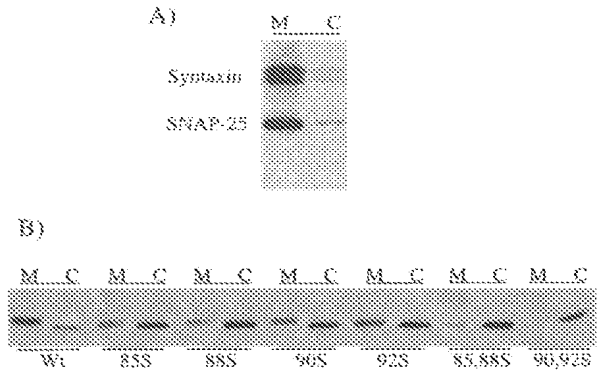

FIG. 4. Membrane association of wild-type and mutant SNAP-25 expressed in COS-7 cells. COS-7 cells were transfected as described in Experimental Procedures. After 48 h, the cells were collected and homogenized on ice and fractionated into membrane and cytosol by centrifugation at 150,000 *g* for 1 h. Equal aliquots of the membrane (M) and cytosolic (C) fractions were loaded for SDS-PAGE and immunoblot analysis. **A:** Subcellular fractionation of rat brain membranes and cytosol showing that syntaxin and SNAP-25 were highly enriched in the membrane fraction. **B:** Distribution of mutant SNAP-25 expressed in COS-7 cells.

turned over, with a half-life of ~3 h. The dynamic palmitoylation and depalmitoylation have been implicated in the regulation of the functions of many proteins including β-adrenergic receptor (O'Dowd et al., 1989; Moffet et al., 1993), neuromodulin (Liu et al., 1993), the GTP-binding protein Gsα (Wedegaertner and Bourne, 1994), and the cation-dependent mannose 6-phosphate receptor (Schweizer et al., 1996). It is, therefore, reasonable to speculate that the functions of SNAP-25, such as its association with membranes and syntaxin, may be modulated through dynamic palmitoylation. Mutations of either $Cys^{85,88}$ or $Cys^{90,92}$ nearly abolished the membrane binding and palmitoylation of the protein (Figs. 2 and 4), suggesting that palmitoylation is necessary, if not sufficient, for the membrane association of SNAP-25. Furthermore, there was a general correlation of the degree of palmitoylation of 85S, 88S, 90S, and 92S with the extent of membrane association (Figs. 2 and 4; Table 2), which supports that the covalently bound palmitate may provide the necessary link between the protein and membranes. Because PC12 cells express several neuronal forms of synaptic proteins such as syntaxin 1A/B, we could not rule out that the SNAP-25 mutants may associate with the membranes via the affinity for membrane-bound synaptic proteins.

Although many palmitoylated proteins have been characterized, no consensus sequences for palmitoylation have been defined. One of the characteristics proposed for the palmitoylation domain is the presence of hydrophobic and positively charged amino acid residues (Liu et al., 1993). The sequence spanning the palmitoylation sites of SNAP-25 is highly hydrophobic and contains three net positive charges (Table 1). Our results suggested that all four Cys of SNAP-25 are required for optimal palmitoylation, and each Cys may be a target for palmitoylation. It is interesting that the palmitoylation appeared to be highly dependent on the presence of multiple Cys residues. Replacing any one of the Cys with Ser led to reduction in the overall incorporation of palmitate ranging from 58 to 90% (Table 2). SNAP-25 sequences from mouse (Oyler et al., 1989), chicken (Catsicas et al., 1991), goldfish (Risinger and Larhammar, 1993), and *Drosophila* and *Torpedo* (Risinger et al., 1993) all contain four to five Cys residues between positions 84 and 92, suggesting the importance of the multiple Cys residues.

The reduced palmitoylation in the mutant SNAP-25 appeared to be due to the increased rate of depalmitoylation by the mutants (Fig. 3). The SNAP-25 palmitoylation in PC12 cells appeared to be turned over more rapidly than that in COS-7 cells (Figs. 1 and 3), probably reflecting the differences in their dynamics of palmitoylation. Although we could not rule out the possibility that the mutation may have affected its structure as a substrate for palmitoyl transferase, the following two lines of evidence suggest that this is a less likely case: (1) Both Cys-to-Ser and Cys-to-Ala mutations showed similar results, and these mutations have been the choice for minimizing disturbance on protein conformations; and (2) the SNAP-25*a* isoform has a switch between Cys^{88} and Phe^{84}, apparently without effect on its palmitoylation (Fig. 1). However, because the present study did not address the question of whether all four Cys residues are palmitoylated under the steady state in neurons, it is necessary to determine the stoichiometry of SNAP-25 palmitoylation by using mass spectrometry before this issue can be resolved.

It is noteworthy that the SNAP-25 polypeptide showed a relatively short half-life of 8–10 h (Fig. 1). Because PC12 cells predominantly express the SNAP-25*a* isoform (Bark et al., 1995), it is possible that the major isoform SNAP-25*b* found in the adult brain has a different turnover rate. This did not appear to be the case because both SNAP-25*a* and SNAP-25*b* in cerebellar neurons cultured for 5 days also showed a rapid turnover rate of ~10–14 h compared with >48 h for syntaxin (J. Sanders, personal communication). The rapid turnover for several synaptic proteins, including synaptotagmin I and VAMP 2, has been documented recently in hippocampal neurons in culture (Daly and Ziff, 1997). It has been proposed that some synaptic proteins are rapidly turned over before synaptogenesis (Daly and Ziff, 1997). The PC12 cells under our experimental conditions did not have synaptic contacts, which may explain the short half-life for SNAP-25.

In summary, the current study demonstrated that SNAP-25 is dynamically palmitoylated, which may regulate its association with the membranes. Furthermore, the rapid turnover of the SNAP-25 polypeptide may play an important role in establishing, maintaining, and strengthening synaptic connections.

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